Soluble Intercellular Adhesion Molecule-1 and E-Selectin Levels in Plasma of Falciparum Malaria Patients and Their Lack of Correlation with Levels of Tumor Necrosis Factor Alpha, Interleukin 1 Alpha (IL-1α), and IL-10

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Intercellular adhesion molecule-1 and E-selectin levels were increased in the plasma of 60 falciparum malaria patients and were not related to levels of tumor necrosis factor alpha, interleukin 10, or interleukin 1 alpha. Soluble E-selectin was correlated to disease; its level in plasma was related to levels of both tumor necrosis factor soluble receptors and biological markers of disease severity and returned to baseline after parasite clearance faster than that of soluble intercellular adhesion molecule-1.

The pathogenesis of malaria is poorly understood; one major question is why some attacks progress to cerebral lesions and death and some others do not. As in many infectious diseases, the host immune response often involves inflammation and deleterious side effects. For malaria patients, high levels of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), have been reported. At least three adhesion molecules, E-selectin (endothelial leukocyte adhesion molecule-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1, are involved in the cytoadherence of parasitized erythrocytes to endothelial cells (EC), leading to obstruction of blood vessels in the brains of patients with cerebral malaria (7). E-selectin is expressed only by cytokine-activated EC. ICAM-1 is expressed by EC, leukocytes, and other cells, and vascular cell adhesion molecule-1 is expressed on the endothelium and epithelium and by macrophages (8). The expression of molecules is up-regulated or induced on the EC surface by IL-1α and TNF-α. All molecules are released in the blood as soluble and biologically active products (for a review, see reference 3). Soluble E-selectin (sE-selectin) and soluble ICAM-1 (sICAM-1) levels are elevated in the plasma of malaria patients, and this increase is not related to outcome (2). To assess whether sICAM-1 and sE-selectin levels in plasma allow monitoring of malaria patients, we focused on the kinetics of these two adhesion molecules. We measured the correlation between sICAM-1 or sE-selectin and TNF-α and its two soluble receptors (TNF-sR55 and TNF-sR75), IL-1α, IL-10 (a potent inhibitor of TNF-α and IL-1), and creatinine and urea (two markers of disease severity).

The study was carried out in Kamenge Hospital, Bujumbura, Burundi. Patients presenting with Plasmodium falciparum infection, as diagnosed with Giemsa-stained blood smears, were enrolled in the study and were given quinine-based therapy for 5 to 7 days. At admission and after 1 and 7 days, blood was drawn and plasma was frozen at −70°C. Forty-four patients were studied again after 14 days. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure the levels of sE-selectin, sICAM-1 (British Biotechnology, Abingdon, United Kingdom), IL-1α (R&D Systems Inc., Minneapolis, Minn.), and TNF-α (Medigenix, Fleurus, Belgium) in plasma. IL-10, TNF-sR55, and TNF-sR75 levels were measured by ELISA as described previously (1, 10). Data were analyzed by the Mann-Whitney and the Spearman rank tests. Sixty adults were enrolled in the study. They had a mean temperature of 39.4 ± 0.1°C and a mean parasite density of 29,822 (95% confidence interval, 16,338 to 54,433) per μl of blood. According to World Health Organization criteria, 12 patients had mild malaria and 48 had severe malaria (including 31 with cerebral malaria). By day 7, seven patients were dead; all survivors had negative blood smears.

At admission, the 60 patients presented with mean (95% confidence interval) levels of 256.5 (205.1 to 320.7) ng/ml for sE-selectin and 412.0 (367.7 to 461.6) ng/ml for sICAM-1 (Table 1). The levels of IL-10 and TNF-α and its two receptors were also increased. Conversely, IL-1α was undetectable in all samples. Among the 60 patients, the levels of sICAM-1 and sE-selectin were not correlated (P = 0.54), and this lack of relation was also observed among the 48 patients with severe malaria (P = 0.58) and the 31 patients with cerebral malaria (P = 0.16). The sE-selectin levels were related to the levels of creatinine, urea, and both TNF soluble receptors (P < 0.003 for all) but not to the levels of TNF-α (P = 0.22) (Table 2). The sICAM-1 levels were not related to the levels of any of these substances (P > 0.26 for all). The levels of both adhesion molecules were correlated, although not significantly, with the levels of IL-10 (P = 0.08) for both. The levels of sE-selectin in plasma were unchanged by day 1, and they returned to normal by day 7 (P = 0.0001) (Fig. 1). The levels of sICAM-1 in plasma remained at high levels after 7 days and reached normal levels by day 14 (P = 0.04).

The increased levels of sICAM-1 and sE-selectin in the plasma of P. falciparum malaria patients, as well as the faster...
TABLE 1. Levels of immunological and biochemical substances in the plasma of 60 patients presenting with falciparum malaria in Burundia

<table>
<thead>
<tr>
<th>Substance (unit)</th>
<th>Mean value (95% confidence interval) for malaria patients</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>256.5 (205.1–320.7)</td>
<td>&lt;96</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>412.0 (367.7–461.6)</td>
<td>&lt;286</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>228.6 (174.3–299.6)</td>
<td>&lt;20</td>
</tr>
<tr>
<td>TNF-sR55 (ng/ml)</td>
<td>5.6 (4.7–6.6)</td>
<td>&lt;3</td>
</tr>
<tr>
<td>TNF-sR75 (ng/ml)</td>
<td>34.9 (28.8–42.4)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>1,339.7 (972.4–1,845.7)</td>
<td>&lt;190</td>
</tr>
<tr>
<td>Creatinine (μmol/liter)</td>
<td>173.9 ± 146.3</td>
<td>53–124</td>
</tr>
<tr>
<td>Urea (mmol/liter)</td>
<td>7.4 (6.4–8.6)</td>
<td>1.7–6.7</td>
</tr>
</tbody>
</table>

* Levels at admission to Kamenge Hospital, Bujumbura, Burundi.

† Values for all substances except creatinine and urea were determined by the mean ± 2 standard deviations of the levels in the plasma of healthy controls (n = 10 to 43).

The kinetics of sE-selectin compared with those of sICAM-1, agree with findings reported for patients with nonsevere malaria (5). sICAM-1 and sE-selectin levels both reflect cellular activation, but they are not related to each other. This may be related to the fact that several of our patients presented with hepatic and renal dysfunction, which alters the clearance of these adhesion molecules. Indeed, the admission levels of sE-selectin correlated with the levels of creatinine and urea in blood. Moreover, 37.5% of patients were coinfected with human immunodeficiency virus type 1, an infection which increases the likelihood of increased sICAM-1 levels (9). However, the levels of both adhesion molecules were similar for patients who were infected with human immunodeficiency virus type 1 and patients who were uninfected.

The concentrations of TNF-α and IL-10 in plasma did not correlate with the levels of circulating adhesion molecules, emphasizing the difference in the kinetics of various molecules reflecting cellular activation. As E-selectin expression is exclusively mediated by TNF-R55 (6), a stronger correlation between sE-selectin and TNF-sR55 levels than between sE-selectin and TNF-sR75 levels was expected. sE-selectin levels correlated with levels of both TNF soluble receptors, but the relation between cell surface expression and the concentrations of these receptors in plasma is unclear. Taken together, our results show that sE-selectin is correlated to disease evolution: sE-selectin returns to baseline levels shortly after parasite clearance, it is related to biological markers of disease severity, but it is not linked to the outcome. As expected, ICAM-1, a molecule with a wider cell distribution, is more likely to reflect an overall cell activation rather than the precise EC status of activation. Elevated levels of sE-selectin in the plasma of P. falciparum malaria patients may reflect an up-regulated expression on EC membranes, leading to increased cytoadherence. Conversely, they may prevent adhesion by the formation of complexes with parasitized erythrocyte ligands. In that respect, cell adhesion molecules could be of major interest for therapy. Antiadhesive therapy, by targeting adhesion molecules with specific monoclonal antibodies, has been reported to be effective against acute inflammatory diseases (4) and murine cerebral malaria (11).

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REFERENCES